

COMPARATIVE PROTEOMIC ANALYSIS OF STEM TISSUE AND XYLEM SAP FROM PIERCE'S DISEASE RESISTANT AND SUSCEPTIBLE GRAPEVINES

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ABSTRACT

Analyses of host plant resistance / susceptibility mechanisms to *Xylella fastidiosa* (*Xf*) infection are critical for understanding host-pathogen interactions. Proteomic analyses of stem tissue and xylem sap samples were initiated to complement genomic approaches employed in elucidating Pierce's disease resistance mechanisms. Samples from one highly resistant and two susceptible grape genotypes were collected at multiple time points post-inoculation from control and *Xf*-inoculated plants. Two-dimensional gel electrophoresis revealed numerous proteins that were differentially expressed and dependent on plant genotype and/or inoculation treatment. Proteins identified by oMALDI-TOF comprised a wide range of functional types. The importance of these proteins with respect to host-pathogen interactions will be investigated further.

INTRODUCTION

While numerous factors including temperature, fertilization and time are known to affect xylem sap chemistry (Andersen and Brodbeck, 1989a, 1989b, 1991; Andersen et al., 1995, 2004b), the protein composition of grape xylem sap in response to *Xylella fastidiosa* (*Xf*) infection has not been investigated in detail to date. In other host plant-pathogen systems, xylem sap proteins were shown to be important in suppression of disease development (Ceccardi et al., 1998; Guo et al., 1993; Nemec, 1995; Reimers and Leach, 1991; Reimers et al., 1992; Rep et al., 2002; Young et al., 1995). Without a doubt, due to its xylem limited growth habit, *Xf*'s growth and development are influenced by xylem sap characteristics. Thus manipulation of the xylem sap composition presents a promising venue to interfere with *Xf* infections.

Disease expression in stems of grape vines results in blocking of water flow to the shoot and, thus, is critical to the lethal nature of Pierce's disease (PD). *Xf* infection results in uneven cane maturation which expresses itself in the formation of green-islands. The irregular nature of green-islands suggests the involvement of localized rather than systemic signals in the formation of the observed spatial symptomology. The importance of *Xf* populations in stems in respect to PD resistance of grape genotypes was also illustrated in recent studies (Krivanek et al., 2005; Krivanek and Walker, 2005). Thus, examination of stem tissue provides an opportunity to identify important aspects of plant-pathogen interactions.

Examination of xylem sap and stem protein makeup is a new approach that allows us to complement our genomic studies conducted on the same susceptible and resistant sibling genotypes employed in this study.

OBJECTIVES

1. Discover xylem sap and stem proteins differentially expressed in PD resistant and susceptible grapes in response to *Xf* infection.
2. Identify differentially expressed proteins from xylem sap and stem induced by *Xf*.

RESULTS

Objective 1. Discovery of differentially expressed proteins.

PD resistant (9621-67) and PD susceptible (9621-94) genetic lines selected from a segregating population of *V. rupestris* x *V. arizonica* as well as *vinifera* grape, Chardonnay were used in this comparative study. We completed expression experiment conducted in the greenhouse where treatment and control grapevines were mechanically inoculated with *Xf* suspension respectively. Leaf and stem tissues were then collected at ten time points from as early as day one post inoculation up to three months when PD symptom was fully expressed in susceptible grapes. A separate set of grapes (same genotypes and treatments as above) was grown in the greenhouse for xylem sap protein extraction at 2 time points post inoculation. The xylem sap was extracted using a pressure chamber following the same sampling scheme as above. Samples (stem, leaf and

xylem sap) collected at each time point were immediately store at -80°C for later protein extraction. After protein extraction, 2-DE separation of protein from all treatments was carried out to characterize differentially expressed proteins.

Xylem sap samples

Two-dimensional gel electrophoresis revealed apparent differential expression of numerous proteins in xylem sap collected from resistant (9621-67) and susceptible (9621-94) genotypes. In addition, infection with *Xf* also appears to affect xylem sap protein expression (Figure 1).

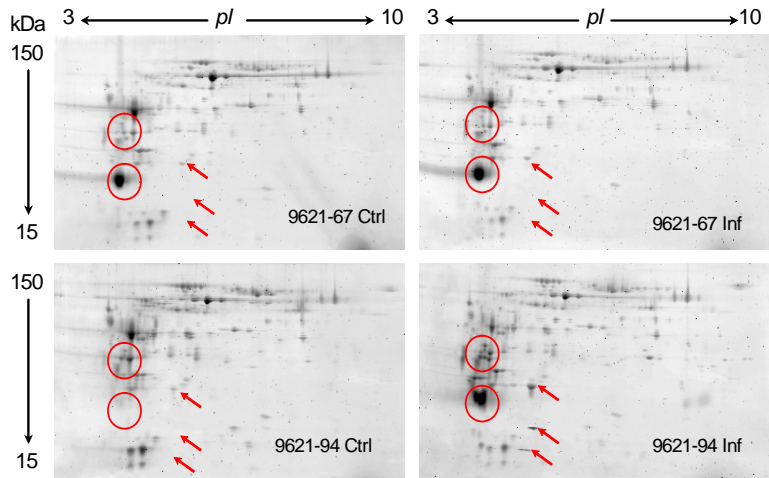
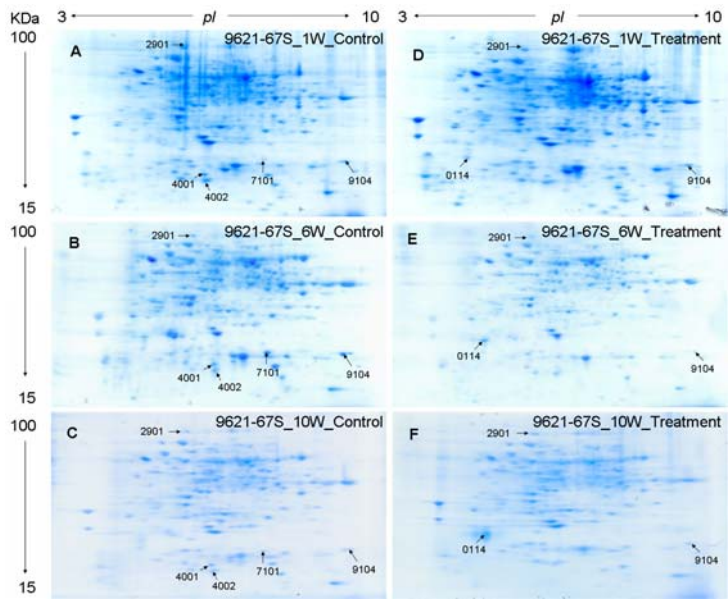


Figure 1. Comparison of the 2-DE gels from xylem sap from the highly resistant (9621-67) and susceptible (9621-94) genotype. Xylem sap samples were collected 6 weeks post-inoculation. Examples of putative differentially expressed proteins are indicated by circles and arrows.

Figure 2. Three gels for each sample from the same stem tissue were run using Bio-Rad 2-DE systems. The resulting 2-DE gel patterns in all three repeats were consistent and reproducible. More than 200 protein spots could be clearly distinguished in the stem protein gels. Approximately 50 of those proteins appear to be differentially expressed (minimum of 2-fold difference in expression). Protein profiles were influenced by genotype, infection status, and stage of disease development (Figure 2). The most profound differences in expression were found in the resistant genotype (9621-67).



Objective 2. Identification of selected proteins.

Twenty-three differentially expressed proteins identified by this approach are listed in Table 1. The identified proteins comprise a wide functional range and their importance in respect to PD pathogenesis / resistance will be investigated in more detail.

Table 1. Differential protein expression was detected between infected and healthy stem tissues in PD resistant and susceptible genotypes. Proteins showing altered expression levels were excised from 2-DE and identified using peptide mass fingerprinting. Database searches using MASCOT algorithms based on the peptide mass fingerprint data identified the differentially expressed proteins.

| SSP No (a) | Score (b) | Masses Matched | Protein <i>Mr/pI</i> | Accession No (c) | Protein description | Seq. cov. % |
|---------------|-----------|-------------------|-------------------------|---------------------|--|----------------|
| 3601 | 440 | 8 | 41915 / 5.25 | gi 11276972 | T51184 actin [imported]-rape | 24 |
| 8702 | 844 | 18 | 57266 / 7.19 | gi 19070130 | AF236127_1 catalase [<i>Vitis vinifera</i>] | 44 |
| 3605 | 284 | 4 | 43516 / 5.81 | gi 18157331 | S-adenosylmethionine synthetase [<i>Phaseolus lunatus</i>] | 13 |
| 2304 | 229 | 3 | 31459 / 4.64 | gi 128207 | Nitrogenase iron protein 2 (Nitrogenase component II) | 18 |
| 4001 | 148 | 5 | 15448 / 5.22 | gi 19114954 | putative transcriptional activator hypothetical protein | 38 |
| 4702 | 112 | 6 | 45160 / 6.09 | gi 127546 | UDP-N-acetylglucosamine enolpyruvyl transferase | 20 |
| 4703 | 201 | 7 | 48823 / 6.33 | gi 9858547 | ribulose biphosphate carboxylase large subunit | 23 |
| 9102 | 234 | 3 | 17104 / 8.40 | gi 729762 | 17.0 kDa class II heat shock protein (HSP 18) | 36 |
| 6002 | 339 | 4 | 17357 / 6.39 | gi 11182124 | pathogenesis-related protein 10 [<i>Vinifera</i>] | 35 |
| 7101 | 423 | 3 | 17813 / 6.92 | gi 2674179 | kinesin-related protein KRP1 [<i>Rattus norvegicus</i>] | 18 |
| 2902 | 164 | 8 | 63122 / 5.19 | gi 861170 | heat shock protein 70 [<i>Zea mays</i>] | 22 |
| 2703 | 356 | 12 | 49249 / 5.19 | gi 4388533 | "F1-ATP synthase, beta subunit [sorghum bicolor]" | 33 |
| 9001 | 280 | 5 | 12602 / 8.42 | gi 8248145 | ORF 109 [<i>Lactococcus lactis</i>] | 49 |
| 7602 | 144 | 4 | 42245 / 6.96 | gi 29125898 | putative nitrous oxide reductase | 20 |
| 7201 | 154 | 4 | 26752 / 6.98 | gi 1708287 | HO_PORPU Heme oxygenase | 19 |
| 3403 | 128 | 3 | 34214 / 5.69 | gi 26397694 | PDXK_ARATH Pyridoxal kinase | 14 |
| 6605 | 199 | 4 | 43542 / 6.44 | gi 23039358 | hypothetical protein [<i>Trichodesmium erythraeum</i> IMS101] | 14 |
| 8506 | 175 | 6 | 37039 / 8.12 | gi 120675 | G3PC_SINAL Glyceraldehyde 3- phosphate | 28 |
| 206 | 117 | 5 | 23353 / 4.71 | gi 9507691 | Putative mobilization protein | 30 |
| 4001 | 197 | 6 | 17935 / 5.01 | gi 29841427 | Similar to NM_025435 RIKEN cDNA 150 | 60 |
| 4002 | 87 | 3 | 16088 / 4.94 | gi 138317 | VGR_BPP1 TailL fiber protein | 24 |
| 9104 | 140 | 7 | 18914 / 8.08 | gi 15896178 | HD superfamily hydrolase [<i>Clostridium acetobutylium</i>] | 29 |
| 7101 | 73 | 4 | 17802 / 7.56 | gi 25091005 | PPIA_HELPJ Peptidyl-prolyl cis- trans isomerase | 32 |

(a) The SSP no. is a number designed by PDQuest Software and is used in matching spots for all gels.

(b) The Score is calculated by the BioAnalyst software

(c) Accession no. according to SWISS-PROT Greater

CONCLUSIONS

Differentially expressed proteins were discovered among genotypes, infection status, and sampling times using a 2-dimensional gel electrophoresis approach. Identification of the differentially expressed proteins is ongoing, however, selected spots were successfully identified using peptide-mass-fingerprinting. The identified proteins fall within a broad range of functional classes, including pathogenesis related proteins. Continued detailed characterization of identified proteins in respect to their potential role in host-pathogen interactions and resistance mechanisms will be necessary to understand their functions and possible utility in controlling PD.

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BREEDING PIERCE'S DISEASE RESISTANT TABLE AND RAISIN GRAPES AND THE DEVELOPMENT OF MARKERS FOR ADDITIONAL SOURCES OF RESISTANCE

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ABSTRACT

This year six crosses for Pierce's disease (PD) resistant table and raisin grapes using seedless parents were made, resulting in 2,398 ovules and 747 embryos. These crosses were based on *Muscadinia* and *V. tiliifolia* sources of resistance. Nineteen seeded crosses consisting of 3,950 emasculations and 49 bagged clusters were made. These crosses were based on *V. arizonica*, *Muscadinia*, and southeastern United States (SEUS) sources of resistance. Over 120 selections have been made based on fruit quality and are ready for greenhouse screening for resistance to PD. Two BC2 families from 89-0908 *V. arizonica* source of resistance segregated in a 1:1 ration for resistance:susceptibility, based on molecular markers associated with the *PdR1* locus. A smaller family from the same source of resistance had an unexpectedly small number of resistant seedlings. DNA samples have been collected from 154 seedlings from the C33-30 x BD5-117 family and are ready for SSR marker analysis. Additional seedlings are being produced to increase probability of identifying markers associated with this source of resistance.

INTRODUCTION

Pierce's disease (PD) has existed in California at least since the late 1800s when it caused an epidemic in Anaheim. A number of vectors for PD already exist in California causing its spread. The introduction of the glassy-winged sharpshooter (GWSS) to California in the 1990's increased the spread and damage caused by PD. Other vectors exist outside California and are always a threat. All of California's table and raisin grape cultivars grown commercially are susceptible to PD. An effective way to combat PD and its vectors is to develop PD resistant varieties so that PD epidemics or new vectors can be easily dealt with. PD resistance exists in a number of *Vitis* species and in *Muscadinia*. PD resistance has been introgressed into grape varieties in the southeastern United States, but fruit quality does not match the *Vitis vinifera* table and raisin grape cultivars grown in California. Greenhouse screening techniques have been improved to expedite the selection of resistant individuals (Krivanek et al. 2005, Krivanek and Walker 2005). Molecular markers have also been identified that make selection of PD resistant individuals from *V. arizonica* in these families even quicker (Krivanek et al. 2006). The USDA, ARS grape breeding program at Parlier, CA has developed elite table and raisin grape cultivars and germplasm with high fruit quality. This collaborative research gives the unique opportunity to develop high quality PD resistant table and raisin grape cultivars for the California grape industry.

OBJECTIVES

1. Develop PD resistant table and raisin grape germplasm/cultivars with fruit quality equivalent to standards of present day cultivars.
2. Develop molecular markers for *Xylella fastidiosa* (Xf)/PD resistance in a family (SEUS) other than those from *V. arizonica*.

RESULTS

Objective 1

This year the seedless embryo culture crosses concentrated on using the *Muscadinia* source of resistance and a unique source of resistance from *V. tiliifolia*. Six crosses were made for a total of 2,398 ovules cultured (Table 1). A total of 748 (31%) embryos were extracted and transplanted on fresh medium for growth into plants. Nineteen seeded crosses were made for PD resistance (Table 2). For five of the crosses, 3,950 emasculations were made and 49 clusters were bagged for 14 additional crosses which had female flowered parents. Fruit has been harvested and seeds are being extracted for germination in January. The number of seeds produced from each resistant source was: 1,881 from *V. arizonica*; 1,643 from *Muscadinia*, and 2,071 from SEUS with an additional 184 from BD5-117 BC1. Over 120 selections have been made based on fruit quality from populations made for PD resistance. These families are from resistant sources different than the *V. arizonica* source of resistance. These selections are in line for PD testing in the greenhouse. Three families (89-0908 *V. arizonica* source of resistance) produced in 2005 were tested for molecular markers associated with *PdR1* locus on chromosome 14